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(54) Physiologically active compositions.

(57) It has previously been shown that bivalent antibodies to insulin can mimic the action of insulin *in vivo*. However antibodies *in vivo* were believed to have the opposite effect.

It has now been found that the administration to vertebrates of certain specific anti-hormone antibodies, preferably monoclonal antibodies, with or without administration of the hormone itself, can potentiate the biological action of the hormone. Active immunisation with a fragment of the hormone can also be carried out. Such methods can be used therapeutically or alternatively to increase the response of the vertebrate to the hormone beyond normal physiological levels. When the hormone is growth hormone, accelerated growth of economically important animals can be achieved.

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Physiologically Active Compositions

This invention relates to hormone activity in vertebrate species.

The term "hormone" has been defined as encompassing any substance released from one part of the body and acting selectively on at least one other, distant, part. Many such substances are secreted by one of the endocrine glands, an example being the secretion of growth hormone from the pituitary gland. The basis for the selectivity for a given target tissue or tissues is the presence or absence in the tissue of receptors which bind the substance specifically. In the case of the protein hormones, such as insulin, the receptors are on the cell surface. Interferon has been implicated in the defence of mammalian cells against viral attack. It is not secreted by an endocrine gland and does not have the specificity of target tissue that, say, growth hormone has. Nevertheless, it is a large glycosylated protein molecule and binds to specific cell surface receptors. There are also certain similar substances which influence the activity of cell by binding to specific receptors on the cell surface, for example tumour necrosis factor and lymphokines such as interleukins. In this specification, the term "hormone" is used to embrace all such protein or polypeptide entities (optionally glycosylated) and the like having a cell-surface receptor.

It has been shown that certain antibodies against insulin and against epidermal growth factor (EGF) potentiate or mimic the activity of those hormones in vitro (Y.Schechter et al, Proc.Nat.Acad.Sci.76(6),2720,(1979) and Y. Schechter et al, Nature 278, 835,(1979)). It is thought that this occurs because the antibodies, being bivalent, cause an aggregation of the hormone-receptor complexes on the cell-surface, such aggregation being involved in activating the second messenger within the cell; monovalent Fab fragments of antibodies do not cause such potentiation or mimicry. These studies have solely been concerned with characterising the insulin and EGF receptors and in identifying the mode of action of such hormones. Furthermore, not all such studies have demonstrated such potentiation (de Pirro et al, Diabetologia 19, 118 (1980) and Schechter et at, Proc. Nat.Acad.Sci, 75(12),5788 (1978)). There has been no suggestion that such potentiation would occur in vivo or that the phenomenon is more widely

applicable. Indeed, the generation of antibodies against insulin and other hormones in vivo was thought to be highly undesirable since the hormone-antibody complexes would be cleared by the body's immune system and, far from being potentiated, the action of the hormone would be negated - see for example Schwartz J., Endocrinology, 107(4),877; Fraisier, Endocrine Reviews 4(2),155 and Gause et al, Endocrinology 112(5),1559 on growth hormone, and Blake & Kelch, Endocrinology 109(6),2175 on luteinising hormone releasing hormone.

It has now surprisingly been found that the administration of certain specific antibodies to hormones can potentiate or mimic the activity of the hormone, provided that the epitope specificity of the antibody is chosen appropriately.

Accordingly, one aspect of the present invention provides a formulation comprising antibodies to a hormone, the epitope specificity of at least some or the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

A second aspect of the invention provides a formulation comprising complexes of (a) a hormone and (b) at least one type of antibody to that hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

A third aspect of the invention provides a method of potentiating or mimicking hormone administration in a "normal" vertebrate (as herein defined) by administering to the vertebrate a formulation comprising antibodies to the hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

The term "normal" is used herein to indicate an individual having sufficient endogenous amount of the hormone in question for normal functioning of the tissues regulated by that hormone.

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A fourth aspect of the invention provides a method of potentiating or mimicking hormone administration in a "normal" vertebrate (as herein defined) by administering to the vertebrate a formulation comprising complexes of (a) the hormone and (b) at least one type of antibody to that hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to the vertebrate, the administration of the hormone in that vertebrate.

A fifth aspect provides a method of treating a human or other vertebrate having abnormally low hormone-regulated tissue function by administering to the vertebrate a pharmaceutical formulation comprising antibodies to the hormone in question, the epitope specificity of at least some or the antibodies being so chosen that the formulation will potentiate or mimic, when administered to the vertebrate, the administration of the hormone in that vertebrate.

A sixth aspect provides a method of treating a vertebrate having an abnormally low hormone-regulated tissue function by administering to the human or animal a pharmaceutical formulation comprising complexes of (a) the hormone in question and (b) at least one type of antibody to that hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

The potentiation or mimicry of the administration of growth hormone, insulin, thyroid stimulating hormone and interferon are particularly preferred aspects of the invention.

The clinical abnormalities which result from a deficiency of a given hormone are in many cases well characterised and will not be listed here. However, examples include (from a deficiency of growth hormone) pituitary dwarfism, Turner's syndrome and cachexia, (from a deficiency of insulin) diabetes and (from a deficiency of thyroid stimulating hormone) cretinism, simple goitre and myxedema.

By antibody "to" a particular hormone, we mean an antibody which will bind to that hormone. Thus, the antibody need not have been created in response to that specific hromone. For example several antibodies raised against growth hormone (GH) will cross-react with chorionic somatomammotropin (CS) because of the extensive sequence homology between the two hormones. Furthermore, it may be possible to raise antibodies to a synthetic analogue or hormone of a portion of it.

It is to be noted that the antibodies need not necessarily be to the specific hormone of the species to which the formulation or method of the invention is being applied. Preferably, however, they are. It has been found that not all antibodies to the hormone will potentiate or mimic the administration of that hormone; instead, the ability of an antibody to act in accordance with the invention appears to depend on the specific determinant (ie. antigenic site) for the antibody on the hormone. It will therefore readily be appreciated that polyclonal antibodies (that is to say, a collection of antibodies having a range of determinant specificities) are less suitable for use in formulations or methods in accordance with the invention, than are monoclonal antibodies. The man skilled in the art will readily, having read this specification, be able by means of routine experimentation to select a monoclonal antibody effective in carrying out the invention. Mixtures of suitable monoclonal antibodies may in some circumstances be used. However, it is nevertheless possible to use animal or human antisera raised by 'conventional' immunization provided that the epitope specificity of the antibodies is as described. Particularly preferred monoclonal antibodies for growth hormone (GH) and chorionic somatomammotropin (CS) are EB01 and EB02. Antibody QB01 is preferred for prolactin (PRL).

It has been found that the presence of the hormone in the animal is necessary for the antibody (when administered alone) to act in the manner described. Thus, in the case of "normal" individuals, administration of the selected antibody alone will have the described effect but, for example, in individuals without endogenous GH, such as pituitary dwarf human children, GH must be administered as well as, but not necessarily simultaneously with, the antibody.

Instead of preparing the antibody outside the animal, it is possible to raise antibodies of the appropriate specificity by injecting the animal with a pre-selected fragment of a suitable growth hormone molecule in combination with an adjuvant. The fragment will be so chosen as to comprise only the epitope or epitopes to which one or more of the hormone-potentiating antibodies are specific and may be derived by cleaving the hormone appropriately, or by synthesising a peptide fragment (or an analogue to such a fragment). By choosing portions of the hormone rich in hydrophilic residues, one is more likely

to be creating or selecting a fragment (an "antigenic determinant") which is on the surface of the complete hormone molecule and which will therefore cross-react with the complete molecule. Equally, the fragment should not contain the site of the hormone which binds to the cell-surface receptor, nor any site which binds a third agent which causes a conformational change such that receptor binding is inhibited. Otherwise, the antibodies produced are likely to inhibit, rather than potentiate,the action of the hormone. Thus, in a successful immunisation of this type, a polyclonal collection of antibodies of narrow specificity is created within the animal in question, thus enabling less frequent injection of the animal than would be the case if exogenous antibodies were passively administered. It will be appreciated that, instead of an actual fragment, a functional fragment could be used, in which the undesirable epitopes of the molecule are present but are shielded from antibody access in some way. The term "fragment" is used in this specification to cover actual and functional fragments.

Accordingly, the present invention also provides a method of increasing a hormone-regulated response of a vertebrate by administering to the vertebrate a preparation comprising at least one pre-selected "fragment" (as herein defined) of an appropriate hormone, optionally in combination with an adjuvant.

The invention also encompasses such a preparation and methods of making such a preparation by conventional means. In such convential vaccines, immunological carriers are frequently used to enhance the immunogenicity of the antigen, for example keyhole limpet haemocyanin or tetanus toxoid. Similarly, adjuvants are often included to stimulate the immune system, for example aluminium hydroxide, saponin or muramyl dipeptide. Generally, about 0.001 to 10μmoles of antigen should be present in a unit dose, preferably about 0.01 to 0.05μmoles, although the selection of a suitable amount of the antigen is well within the capabilities of one skilled in the art.

In the case of passive transfer of antibodies to a vertebrate, approximately 10^4-10^7, preferably 10^5-10^6 ABT_{50} units of antibodies should be administered in any suitable sterile medium, such as saline, to give a dose of 0.01 to 10ml, preferably about 0.5ml.

To take only three hormones as an example, namely GH, CS and PRL,

formulations or methods in accordance with the invention are believed to offer potential in:

(a) accelerating the attainment of full growth of industrially important (ie. farmed) animals such as cattle, pigs and poultry or achieving such growth on reduced amounts of feed;

(b) increasing the growth of such animals beyond the normal maximum;

(c) increasing the duration or extent of lactation in mammals, for example to obtain a greater milk yield from cattle or to enable a human mother to breast-feed an infant;

(d) increasing the proportion of lean meat to fat in farmed animals;

(e) increasing the growth of fleece, fur or other useful surface products of animals, for example sheep;

(f) treating a GH-deficient individual, for example a dwarf child, to enable normal growth to occur.

In all cases, it is believed that the use of formulations and/or methods in accordance with the invention may offer significant cost-saving and labour-saving advantages in comparison with the use of the hormone alone, not least because the potentiation of the hormone action is expected to result in fewer administrations being needed. Furthermore, a reduction of possibly harmful residues in the meat or milk of treated animals may be expected. Finally, because farmed animals are frequently routinely injected with other vaccines, for example against foot and mouth disease, it would be extremely convenient to incorporate in such a vaccine a formulation in accordance with the present invention.

The invention will now be described by way of the following non-limiting Examples.

Abbreviations

LIST OF ABBREVIATIONS

hGH	-	human growth hormone
hCS	-	human chorionic somatomammotropin
hPRL	-	human prolactin
bGH	-	bovine growth hormone
MAB	-	monoclonal antibody
PBS	-	phosphate-bufferred saline
PMSF	-	phenyl methyl sulphonyl fluoride
Ig	-	immunoglobulin
MHC	-	major histocompatibility complex
SPRIA	-	solid phase radioimmunoassay
W_{to}	-	weight at time 0
SDS-PAGE	-	SDS polyacrylamide gel electrophoresis
EDTA	-	ethylene diamine tetracetic acid
RIA	-	radio-immuno assay

Statistical evaluation

Arithmetic means and standard deviation values were calculated using conventional methods. Differences between groups were assessed by unpaired Student's t-test.

Description of Figures

Figure 1 relates to Example A and shows weight gain in dwarf mice with compositions in accordance with the invention;

Figure 2 relates to Example C and shows corresponding weight gain in normal mice;

Figure 3 relates to Example D and shows the weight gain of the pigeon crop sac; and

Figure 4 relates to Example E and shows the weight gain of marmosets.

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PREPARATIVE EXAMPLES

Example 1: Preparation of Monoclonal Antibody to Human Growth Hormone (hGH).

The antibodies employed in this study are available from Wellcome Diagnostics, Temple Hill, Dartford, Kent. U.K. and have been characterized extensively (Ivanyi, 1982 a b, Aston and Ivanyi, 1983). BALB/c mouse spleen cells were fused with NSI myeloma cells and cloned by standard techniques (Ivanyi and Davis, 1980, 1981). The antibodies derived were all of the IgG_1 isotype and were non-precipitating when examined by double diffusion in agar. Four determinants have been defined on hGH by competition assays (QA68, NA71, EBO1 and EBO2) of which two are completely shared with hCS (EBO1 and EBO2). However, none of the antibodies cross-reacted with human prolactin. Antibody concentrations have been expressed as ABT_{50} values which correspond to the reciprocal antibody titre required to give 50 percent binding of ^{125}I-hGH by RIA (Ivanyi, 1982a). Binding studies with proteolytically modified forms of hGH suggest that all four determinants are located in the first 1-139 redidues with the EBO1 determinant also represented in the sequence region 146 - 191 (Aston and Ivanyi, 1983).

Example 2:

PREPARATION OF Fab' FRAGMENT OF EBO1

Ascitic globulin (5mg/ml) of EBO1 was affinity purified on hGH (100mg) immobilized on CNBr-activated Sepharose. Retained material was eluted with glycine-HCl buffer pH 2.3 and tubes containing protein material were immediately adjusted to pH 7.5 with NaOH(1M). The purified antibody was concentrated to 20mg/ml (2ml) and dialysed against sodium phosphate buffer (0.5M, pH 8.0) containing cysteine (0.01M) and EDTA (.002M).

This material was digested with 0.4mg of papain (BDH) for 4 hours at 37°C followed by dialysis against PBS to remove the cysteine and EDTA.
Subsequently, the dialysate was applied to a column of DEAE cellulose (20cm x 1.2cm) and eluted with a linear gradient consisting of sodium phosphate buffer (0.005M - 0.3M, pH 8.0). The first peak to be eluted from the column contained no

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antibody heavy chain as determined by SDS-PAGE and retained an activity of 10^{-3} x ABT_{50}.

Example 3: HORMONES

Human growth hormone employed for injection was derived from stocks of out-dated clinical grade material obtained by special agreement with the Institute of Child Health, London, whereas hormone used in assays was of >99% purity. hGH is avaiable from RIA(UK) Ltd, Washington, Co. Durham U.K. Radioiodination of hGH was performed with lactoperoxidase resulting in a tracer of high specific activity (80×10^6 cpm/ g) (Linde et al, 1981). Monomeric ^{125}I-hGH was separated from any aggregated material prior to assay by Ultrogel column chromatography. Ultrogel is a trade mark of LKB Ltd, Cambridge, U.K. Antibody-hormone complexes for administration into animals were prepared by mixing the solutions for 1 hour prior to injection. In chronic experiments, where injections were given for several weeks, the complexes were prepared in batches enough for 1 week and stored at ±4°C.

A soluble extract was prepared from the marmoset pituitary gland by homogenizing the tissue in 0.05M sodium bicarbonate 2mM PMSF pH 8.6. The resulting homogenate was centrifuged at 10,000g for 20 minutes and the supernatant (20ml) was tested.

BIOLOGICAL EXAMPLES

Example A: CUMULATIVE WEIGHT GAINS IN hGH-EBO1 COMPLEX TREATED DWARF MICE

Dwarf mice were bred from normal animals heterozygous for the dw gene or from a heterozygous female mouse and a male homozygous dwarf mouse treated with thyroxine. The dwarf mice, weighing 9.1± 0.4g, were allocated at random to treatment groups of six animals and then distributed among several small cages each containing one representative of each treatment group. Hormones were

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injected subcutaneously in the back in 0.1ml, for the periods indicated. Weights were measured at the onset, during and sometimes after treatment. Weight gains in short-term experiments or the cumulative weight gains over several days of study were expressed as relative values (%) related to initial whole body weights or as net weights (g). Tail lengths were measured by the method of Hughes and Tanner (1970).

Over a three week period, control mice, treated with phosphate-buffered saline (PBS), increased their relative weight gains by about 15% (Figure 1). Mice treated with 10 μg hGH gained 22% over starting weight over the same period. However, hGH-EBO1 complexes raised the cumulative weight gain to 34%, which corresponds to an additional 12% increment over that achieved from treatment with hGH only.

Raising the hGH dose in the complex from 10 μg to 160 μg, increased the weight gains to 44% of the initial body weight. It is apparent (Figure 1) that the differences between treated groups and controls progressively increased over the 21 day test period. Whilst hGH-MAB complexes produced a significant increment within 48h, the difference between the group treated with hGH only and the PBS-injected control group was not apparent until day 7.

Example B: GROWTH POTENTIATION BY EBO1 Fab' FRAGMENTS

In order to assess whether the bivalency of EBO1 antibody was a pre-requisite for growth potentiation, complexes of EBO1-Fab' -hGH were examined for their effects on $^{35}SO_4^{2-}$ uptake in dwarf mice.

Dwarf mice within a relatively narrow weight range (7 - 10g) were randomized by use of tables of random numbers (Fisher and Yates, 1957) and injected with a dose of $^{35}SO_4^{2-}$ related to body weight (0.5 Ci/g body weight) 24h after the final hormone injection (Herbal, 1970). Mice were killed 20h later when rib cages were removed, placed in boiling water for 20 min, soaked overnight in saturated sodium sulphate and washed in tap water for 2 h and distilled water for 1 h. The bony

portion of ribs together with about 1 mm of adjacent rib cartilage (costochondral junction) was then cut away leaving the costal cartilages attached to the sternum. All adhering soft tissue was removed and the five longest costal cartilages which articulated directly with the sternum were detached whole from each side of the rib cage and combined for each animal. Each pool of ten costal cartilages was then dried at room temperature overnight, weighed and processed for the measurement of $^{35}SO_4^{2-}$. The uptake of $^{35}SO_4^{2-}$ by costal cartilage was expressed as disintegrations per minute per mg of cartilage.

The levels of sulphate uptake by cartilage potentiated by EBO1 or EBO1-Fab' were not significantly different when comparing the antibody and fragments at the same ABT_{50} dose (Table 1). However, the growth observed in the presence of hGH alone was significantly less than that observed for Fab' -hGH.Increasing the valency of the Fab' fragment by including a "second" either monoclonal anti-light chain antibody (TC187) or polyclonal anti-mouse Ig antibody did not significantly alter the $^{35}SO_4^{2-}$ uptake. Furthermore, Fab' fragments did not competitively inhibit the potentiating effect of EBO1 antibody. Preparation of complexes of EBO1 with equimolar quantities of hGH and hCS also did not decrease the degree of potentiation. Such complexes would comprise mainly the species hGH-EBO1-hCS which would be expected to have decreased activity if bivalency was necessary, since hCS has only 10% somatotropic activity of hGH. Indeed, the EBO1-hCS complex resulted in significantly lower growth activity than the corresponding complex with hGH.

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TABLE 1.

THE EFFECT OF ANTIBODY Fab FRAGMENT-hGH COMPLEX ON SULPHATE UPTAKE ACTIVITY

HORMONE (μg)	ANTIBODY (μg)		$^{35}SO_4^{2-}$ uptake dpm/mg ± SD
	EBO1	ANTI MOUSE Ig	
hGH(160)	-	-	1640 ± 160
hGH(160)	Ig(200)	-	4280 ± 300
hGH(160)	Ig(20)	-	2500 ± 400
hGH(160)	Fab(20)	-	2700 ± 120*
hGH(160)	Fab(20)	TC187(12)	2300 ± 100
hGH(160)	Fab(20)	R-Poly(5)	2000 ± 100
hGH(160)	Fab(20)+Ig(200)	-	4880 ± 190
hCS(160)	Ig(200)	-	1200 ± 70
hGH(80)+hCS(80)	Ig(200)	-	3820 ± 250
hGH(80)+hCS(80)	Ig(200)	R-Poly(5)	4250 ± 500
PBS	-	-	600 ± 75

Dwarf mice (n = 6) were injected twice with hormone plus antibody (0 and 24h) followed by $^{35}SO_4^{2-}$ (48h) and harvested at 72h.

TC187 = Rat monoclonal anti-mouse L-chain; R-Poly = rabbit polyclonal anti-mouse Ig.

*$p<.001$ compared with hGH alone.

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Example C: POTENTIATION OF GROWTH IN JUVENILE BALB/c MICE

Since the stimulation of growth in dwarf mice is exercised over a background of very slow activity, it was of interest to ascertain if the antibody mediated potentiation effect could be demonstrated in normally, i.e. rapidly, growing juvenile mice. Three week old BALB/c mice weighing between 7 - 10g were randomized and injected as described above for dwarf mice. Weights were taken 3 times/week in chronic experiments. Group 1 received PBS twice weekly throughout; Group 2 had 160μg hGH three times over 1 week and Group 3 the same thrice weekly for 4 weeks; Groups 4 and 5 had complexed 160μg hGH/200μg EBO1 thrice weekly for 1 or 4 weeks respectively; and Group 6 had daily injections of the said complex for 4 weeks. Groups 5 and 6 grew by 31% and 37% more than PBS injected controls (Fig. 2). A significant weight gain effect of hGH-EBO1 complexes was apparent as early as 48 hours after administration (Table 2). Animals receiving 10, 40 or 160μg of hGH in the presence of EBO1 demonstrated a significant weight gain increment when compared with hGH only.

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TABLE 2.

SHORT-TERM WEIGHT GAINS IN JUVENILE BALB/c MICE INJECTED WITH VARIOUS DOSES OF hGH COMPLEXED WITH EB01 ANTIBODY

hGH µg	hGH-EB01 Complex (160/200µg)	Relative Weight Gain % ± SD	p (significance)
-	-	13.0 ± 11.0	
10	-	16.0 ± 12.0	<0.050
	+	27.0 ± 6.0	
40	-	16.0 ± 11.0	<0.050
	+	29.0 ± 8.0	
160	-	13.0 ± 13.0	<0.025
	+	29.0 ± 11.0	

Three week old mice (W_{to}=8.0 ± 1.0) were injected at time 0 and 24h.Weight gains, expressed as % of body weight, were determined at 48h.

Example D: POTENTIATION OF THE LACTOGENIC ACTIVITY OF hGH

Human growth hormone produces significant lactogenic activity as measured by its effects on pigeon crop-sac or mammary tissue in vivo and by its ability to displace ^{125}I-hPRL from binding to mammary gland receptors. The pigeon crop-sac bioassay procedure measures the lactogenic activity of hormones and is analogous to other mammotropic assays involving either mammary gland or corpus luteum of rats or mice. Hormones, complexes or control solutions were administered (0.1ml) from coded vials intradermally adjacent to each hemicrop, there being five birds in each group. The injection protocol was either one administration on day 1 only ($2X10^4$ ABT_{50}) or three over 36 hours (each of $2X10^3$ ABT_{50}) or combined with two

further injections on day 2. Birds were killed on day 3 and the wet weight of the crop-sac mucosa of 2.5 cm diameter was determined (Nicoll, 1967). By administering the complex or free hormone intradermally, adjecent to each of the two individual hemicrops, the potentiation effect has been examined under conditions which excluded systemic hormone distribution. The weight of the crop-sac mucosa following the injection of 100 μg of hGH in three doses was about 100mg, whereas the control (PBS or antibody only treated) mucosa weighed 10 - 13mg (Figure 3).

Treatment with 10 μg of hormone alone produced a mucosa of 48mg, but in the presence of EBO1 or EBO2 the mucosal weight increased to 108mg and 80mg respectively. As in dwarf mice, NA71 was without potentiating activity whereas QA68 significantly depressed the mucosal secretion. Furthermore, EBO1 potentiated the lactogenic effects proportionally by the same extent whether the hormone was administered in one or three doses. However, EBO1 failed to potentiate the lactogenic effect of saturating doses of hGH. Since EBO1 binds equally to hCS, we also examined the potentiation of the lactogenic activity of this hormone. The results show that antibody complexes with 10 μg hCS had doubled the weight of the crop-sac mucosa in comparison with controls receiving the hormone alone.

Example E: POTENTIATION OF GROWTH IN MARMOSETS

The potentiation effect in either murine growth or pigeon crop-sac responses was dependent on the administration of exogenous hGH since none of the antibodies described here cross-reacted with the rodent growth hormone. However, we discovered that EBO1 antibody did bind to marmoset growth hormone when tested by immunoblot assay. This observation enabled the assessment of EBO1 potentiation of marmoset growth in the absence of any exogenously administered hormone (Figure 4). Sixteen animals, randomised on a weight basis, were divided into four groups receiving 0.4mg hGH only, hGH (0.4mg) + EBO1 (2mg), EBO1 (2mg) only or PBS three times per week. Two sister animals had to be removed from the experiment after 1 week due to continuous weight loss. Animals treated for 44

days with PBS increased their weight by 68g, whereas the group receiving EBQ1 only demonstrated a mean weight increase of about 103g. Groups receiving hormone only or complex had weight gains intermediate to those observed with EBO1 only and the control group. The relative weight gain (% over initial body weight) of animals receiving antibody was 89% whilst the control group increased their body weights by only 61%. Despite continuous administration of heterologous antibody to the marmosets for 6 weeks, side effects, possibly of anaphylactic origin, have so far not been observed.

Example F: FURTHER PIGEON CROP SAC ASSAY

The pigeon crop sac assay of Example D was repeated with 1 μg of highly purified (prep. L) or 3 μg of QBO1-MAB affinity purified (prep. R) hPRL alone or in complex with a constant dose, 2500 ABT_{50} of monoclonal antibodies. The preparation of QIBO1 antibodies was analogous to that described in Example 1 above and has been published (Ivanyi & Davies, 1981).

The results, given in Table 3, show strong potentiation for antibody QBO1, for both preparations of hPRL. The figures represent mean values for six crops per group ± one standard deviation.

TABLE 3:

Antibody	Weight in Crop Sac (mg)	
	Prepn. L	Prepn. R
QBO1	68.0 ± 30.5	75.0 ± 18.0
Control A (hPRL alone)	22.5 ± 14.5	22.0 ± 11.0
Control B (uninjected)	19.5 ± 5.0	

Example G: Dwarf Mouse Body Growth and Composition

Dwarf (dw/dw) mice were divided into three groups of eighteen and fed on 100%, 75% or 50% of the usual ad lib consumption for 10 days. Within each diet, six mice were treated with saline, six with hGH (40 milliunits) and six with hGH/EB01 ($10^4 ABT_{50}$ of antibody) complex. The mice were assessed for overall growth ($^{35}SO_4$ uptake into intercostal cartilage, sulphate injected at day nine), fat content, weight change and length of tail. The results are presented in Tables 4 to 7.

It is apparent that the use of a hormone/antibody complex can compensate for a reduced diet and can also reduce the proportion of fat in an animal by preferentially causing growth of muscle, the latter advantage being even more marked with a reduced diet.

TABLE 4: Sulphate Uptake into Intercostal Cartilage

	DIET		
	100%	75%	50%
Treatment			
Saline	2393± 315	695± 76	540± 57
hGH	5569± 209	4492± 317	2958± 202
hGH+ EB01	7956± 865	5531± 691	4840± 668

The results are expressed as disintegrations per minute per milligram of tissue; mean ± S.E.

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TABLE 5: Fat Content

	DIET		
	100%	75%	50%
Saline	2533 ±83	1502 ±304	1082 ±89
hGH	1857 ±130	1173 ±127	631 ±152
hGH+ EB01	1164 ±99	733 ±51	347 ±91

Figures are amounts of fat in milligrams

TABLE 6: Weight Change as a % of Initial Weight (initial weight = 100%)

	DIET		
Treatment	100%	75%	50%
Saline	104.67± 0.95	94.5± 1.63	87.83± 1.14
hGH	119.67± 1.94	107.5± 1.61	91.67± 0.8
hGH+ EB01	127.17± 2.12	111.67± 0.88	95.2± 2.52

Figure expressed as mean ± S.E.

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TABLE 7: Longitudinal Growth of Tail (Increase in mm).

Treatment	DIET		
	100%	75%	50%
Saline	0.96 ± 0.24	0.5± 0.19	0.71± 0.36
hGH	3.33± 0.3	3.29± 0.47	2.04± 0.28
hGH+ EB01	5.21± 0.39	4.96± 0.31	4.05± 0.16

Figure expressed as mean ± S.E.

Example H: Growth in Normal Mice

The experiment of Example C was repeated, with the assay being for $^{35}SO_4$ uptake into intercostal cartilage rather than weight gain. Mice aged 4 weeks (weight 10g), 6 weeks (14g) and 9 weeks (19g) were used and injected sub-cutaneously with 0.1ml of either saline, hGH (100μg) or hGH-EB01 complexes (100 μg/10^4ABT$_{50}$) two days before administration of $^{35}SO_4$. Cartilage was removed 24h later. The results are given in Table 8 and shown significantly increased growth with the complexes ($p<0.0005$ for the oldest mice).

TABLE 8:

Treatment	MICE AGE		
	4 weeks	6 weeks	9 weeks
Saline	1686±282	1176±85	505±32
hGH	1621±158	1330±83	605±54
hGH/EB01	2431±307	1738±191	1071±85

Units: counts/min/mg.cartilage

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Example I: Use of Polyclonal Antibodies of Reduced Specificity

A 7K (7000 Daltons) fragment was cleaved from the C-terminal of hGH with subtilisin followed by chromatography under denaturing conditions (Aston & Ivanyi 1983). Two mice were each injected with 50μg of the fragment emulsified with Freund's complete adjuvant but without a carrier and 21 days later received a further 50μg without any adjuvant. Serum was taken 10 days after the second challenge, complexed with hGH (10μg) and injected into dwarf mice. The subsequent growth of mice was assayed by measuring $^{35}SO_4$ uptake as above. The results (Table 9) show that the polyclonal antiserum potentiated growth.

TABLE 9:

Treatment	$^{35}SO_4$ uptake (c.p.m./mg.cartilage)
Saline	500±50
hGH (10μg)	1365±146
hGH plus antiserum I	4425±703
hGH plus antiserum II	3272±471

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Example J: Potentiation of Ovine Growth Hormone Activity

Monoclonal antibodies to ovine (ie. sheep) growth hormone (oGH) were prepared in an analogous way to the method of Example 1, and are available from the Department of Experimental Immunobiology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, UK. Dwarf mice, which respond to oGH, were divided into groups of six and treated with 50μg of the hormone, either alone or complexed with one of four such antibodies, on two consecutive days prior to injection with $^{35}SO_4$. Intercostal cartilage was removed 24 hours later, dissolved in formic acid and the radioactivity counted. The results are given in Table 1:

Table 10

Treatment	MAB titre -	Uptake of $^{35}SO_4$ (mean ± S.E.; cpm/mg tissue)
oGH alone	-	1411 ± 261
oGH + 1D11 H9	$1X10^{-4}$	5871 ± 1339
0GH + 4B62 D9	$3.2X10^{-4}$	4323 ± 671
oGH + 2B11	$4.2X10^{-3}$	3408 ± 642
oGH + 3B11	$5.6X10^{-2}$	3434 ± 719
Saline	-	557 ± 79

Example K: Potentiation of Growth in Sheep

Groups of two sheep (mean weight 17kg) were treated with differing doses of anti-oGH antibody 2B11 (see Example J) or, as a control, mouse globulin, on two consecutive days before intraperitoneal injection of $^{35}SO_4$ (146μCi/kg). Quadruplicate samples of intercostal cartilage were removed from each site 24 hours later and analysed as above. The results are given in Table II:

Table II

Treatment	Incorporation of $^{35}SO_4$ (mean ± S.D.; cpm/10mg cartilage)
10^6 ABT_{50} 2B11	3094 ± 630
$0.2X10^6ABT_{50}$2B11	5168 ± 24
$0.04X10^6ABT_{50}$2B11	2373 ± 183
Control	2084 ± 771

These results, which are highly significant by variance analysis, show that formulations in accordance with the invention can potentiate the action of endogenous GH in an economically important species.

Example L: Potentiation of Growth in Sheep

Example K was repeated additionally using a different monoclonal antibody, 1D11 H9, and groups of five sheep, mean weight 24 kg. The results are given in Table 12:

Table 12

Treatment	$^{35}SO_4$ Uptake (mean ± S.E.; cpm/10mg)
$8.8X10^5 ABT_{50} 2B11$	3315 ± 560
$2X10^6 ABT_{50} 1D11$ H9	2818 ± 343
control immunoglobulin	1908 ± 299

Significantly ($p<0.05$) increased growth is seen with the MAB-treated groups.

Example M: Potentiation of Thyroid Stimulating Hormone (TSH) Activity

TSH is a glycoprotein produced by the pituitary gland and activating the thyroid gland in vertebrates. A deficiency of TSH causes involution of the thyroid gland and flattening of the epithelium. In humans, such a deficiency can be responsible for cretinism, simple goitre and the panoply of abnormal conditions known collectively as myxedema. It may be treated with iodine compounds or thyroid gland extracts. Dwarf mice are hypopituitary and the thyroid gland is involuted. Treatment with TSH raises the serum T_4 levels and causes some histological repair of the thyroid.

A monoclonal antibody (GC73) to TSH was prepared analogously to those of Example I above and is available from the same address as in Example J. GC73 is specific for the β-chain of TSH. Dwarf mice were randomly divided into groups of five and treated accordingly to the regimes of Table 13 on five consecutive days before analysis of the serum for T_4 level by radioimmunoassay, and microscopic histological inspection of thyroid tissue, fixed in 10% formalin in saline solution and then embedded in wax or plastic. The T_4 data are given in Table 13; the results were confirmed by microscopic examination.

Table 13

Treatment	T_4 level (mean ± S.E.)
0.1 international units TSH	62.8 ± 3.7
0.05 units TSH	36.4 ± 6.3
0.1 units TSH + $10^4 ABT_{50} GC73$	116.2 ± 5.3
0.05 units TSH + $10^4 ABT_{50} GC73$	72.2 ± 11.75
Saline	less than 5
$10^4 ABT_{50} GC73$ only	less than 5

CLAIMS

1. A formulation comprising a pharmaceutically acceptable carrier and antibodies to a hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

2. A formulation comprising a pharmaceutically acceptable carrier and complexes of (a) a hormone and (b) at least one type of antibody to that hormone, the epitope specificity of at least some of the antibodies being so chosen that the formulation will potentiate or mimic, when administered to a vertebrate, the administration of the hormone in that vertebrate.

3. A formulation according to claim 1 or 2 wherein the hormone is growth hormone, prolaction or chorionic somatomammotropin.

4. A formulation according to claim 3 wherein the hormone is growth hormone.

5. A formulation according to any one of the preceding claims wherein the antibody is a monoclonal antibody.

6. A formulation according to claim 4 wherein the antibody is a monoclonal antibody having substantially the same epitope specificity as EB01 or EB02.

7. A method of potentiating or mimicking hormone administration in a "normal" vertebrate (as herein defined) by administering to the vertebrate a formulation according to any one of the preceding claims.

8. A method of increasing a hormone-regulated response of a "normal" vertebrate (as herein defined) by administering to the vertebrate a preparation comprising a least one pre-selected "fragment" (as herein defined) of an appropriate hormone, optionally in combination with an adjuvant.

9. A method according to claim 8 wherein the response is regulated by growth hormone.

10. A formulation comprising a "fragment" (as herein defined) of a hormone and/or an immunological carrier and/or an adjuvant, in combination with a pharmaceutically acceptable carrier.

11. A process for preparing a formulation according to claim 1 comprising the steps of

(a) preparing a monoclonal antibody to the said hormone of restricted specificity,

(b) determining whether the antibody from step (a) potentiates or mimics the activity of the hormone and, if it does,

(c) bringing the antibody into association with a pharmaceutically acceptable carrier.

12. A process according to claim 11 for preparing a formulation according to claim 2, wherein, after step (b) and before step (c), the antibody is complexed with the said hormone.

13. A formulation according to any one of claims 1 to 6 and 10 for use as a medicament.

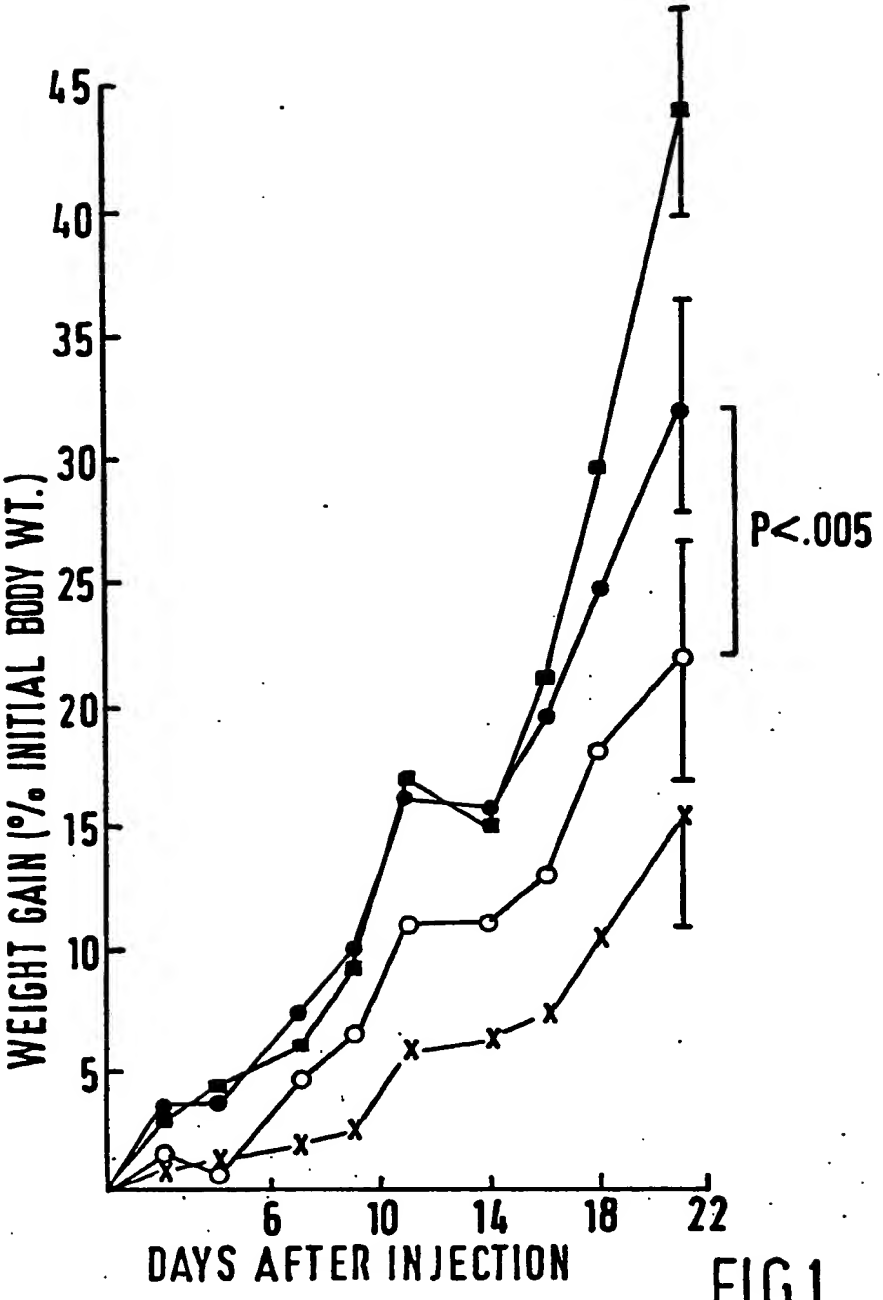
45
40
35
30
25
20
15
10
5
WEIGHT GAIN (% INITIAL BODY WT.)
P<.005
6
10
14
18
22
DAYS AFTER INJECTION
x-x PBS(control)
o-o hGH
●-● hGH-EB01 (10μg/200μg)
■-■ " (160μg/200μg)

FIG.1

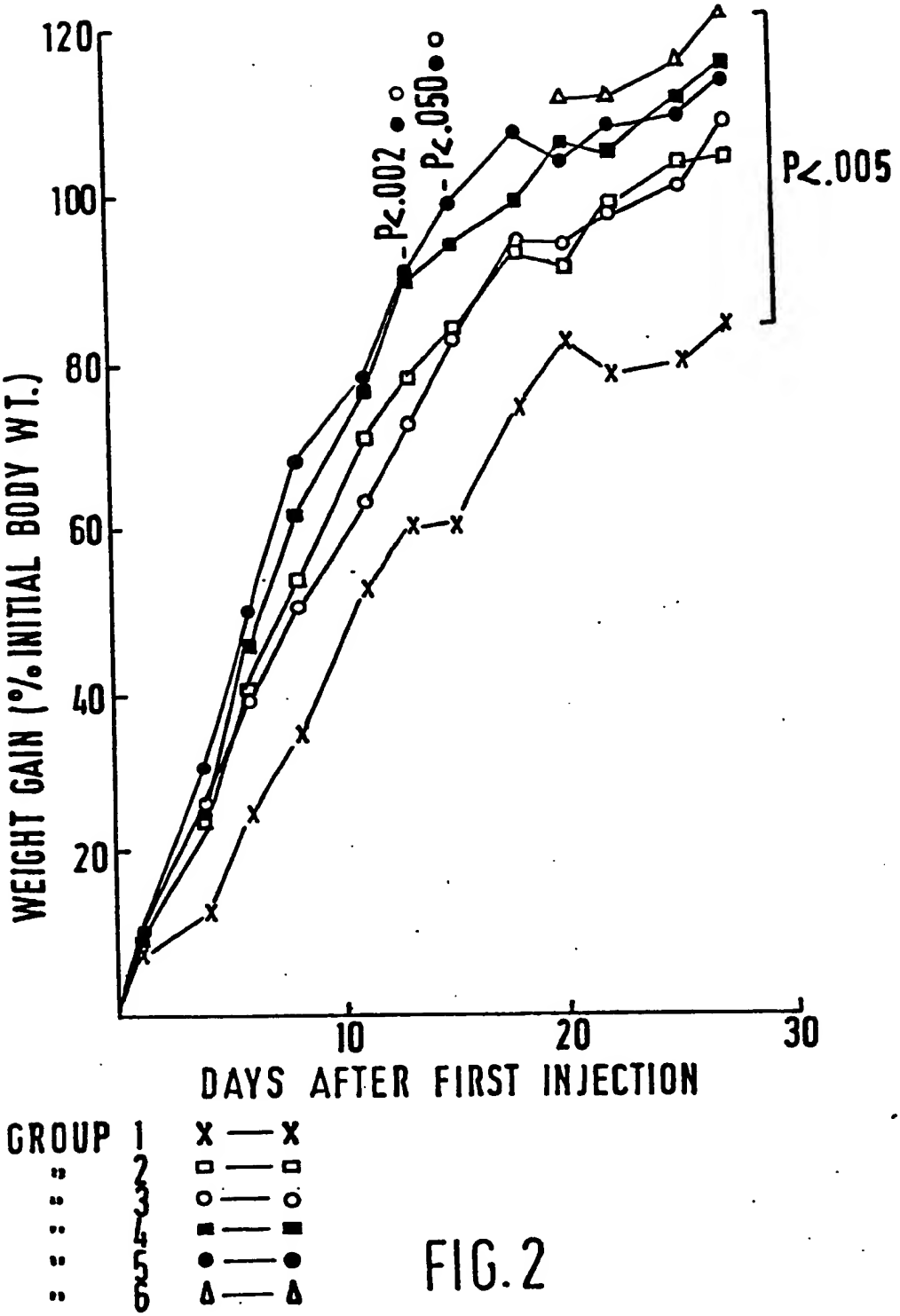
120
100
80
60
40
20
WEIGHT GAIN (% INITIAL BODY WT.)
P<.002
P<.050
P<.005
10
20
30
DAYS AFTER FIRST INJECTION
GROUP 1
2
3
4
5

FIG. 2

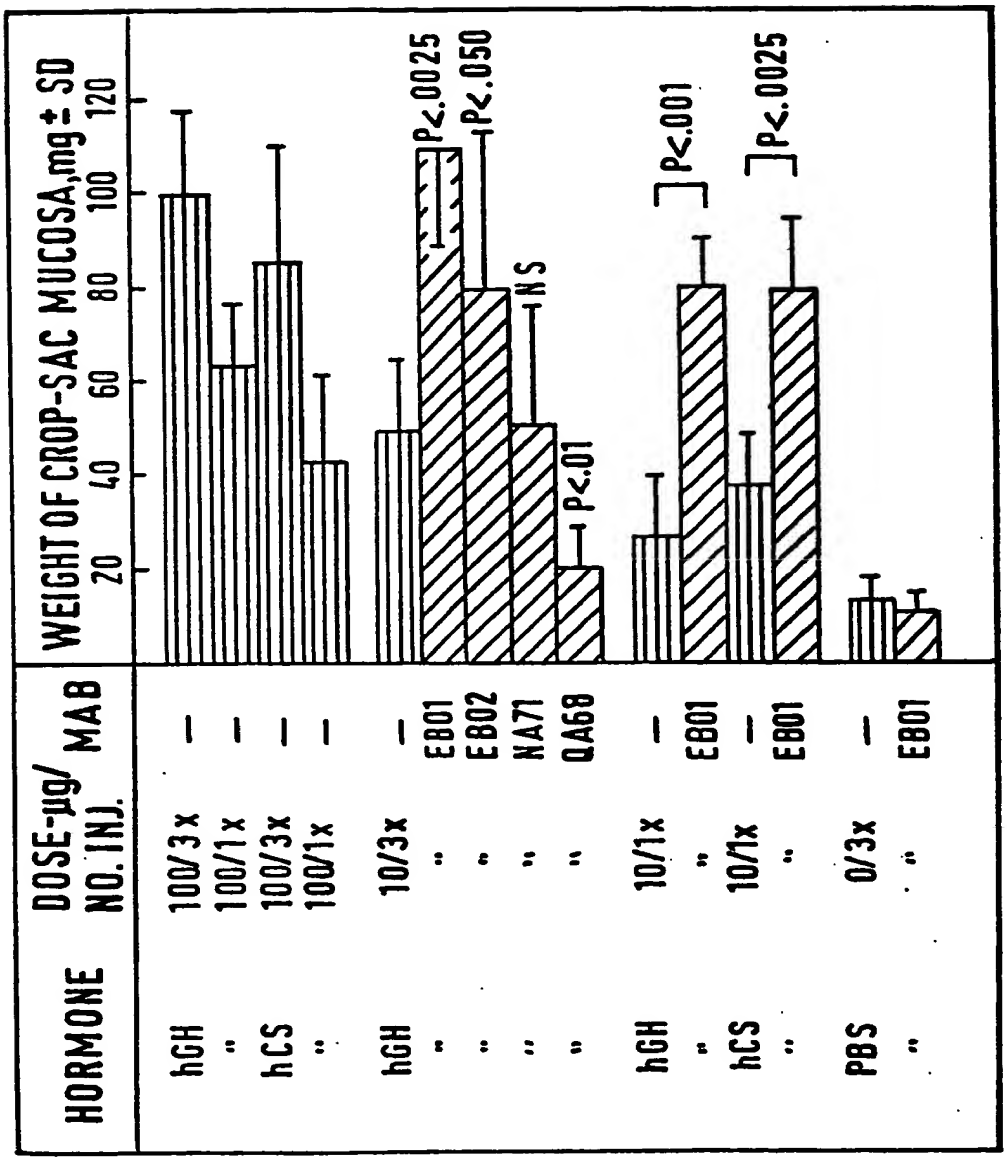
HORMONE
DOSE-µg/ NO. INJ.
MAB
hGH 100/3x —
" 100/1x —
hCS 100/3x —
" 100/1x —
hGH 10/3x —
" " EB01
" " EB02
" " NA71
" " QA68
hGH 10/1x —
" " EB01
hCS 10/1x —
" " EB01
PBS 0/3x —
" " EB01
WEIGHT OF CROP-SAC MUCOSA, mg ± SD
20 40 60 80 100 120
P<.0025
P<.050
NS
P<.01
P<.001
P<.0025
WITH MAB
WITHOUT MAB

FIG.3

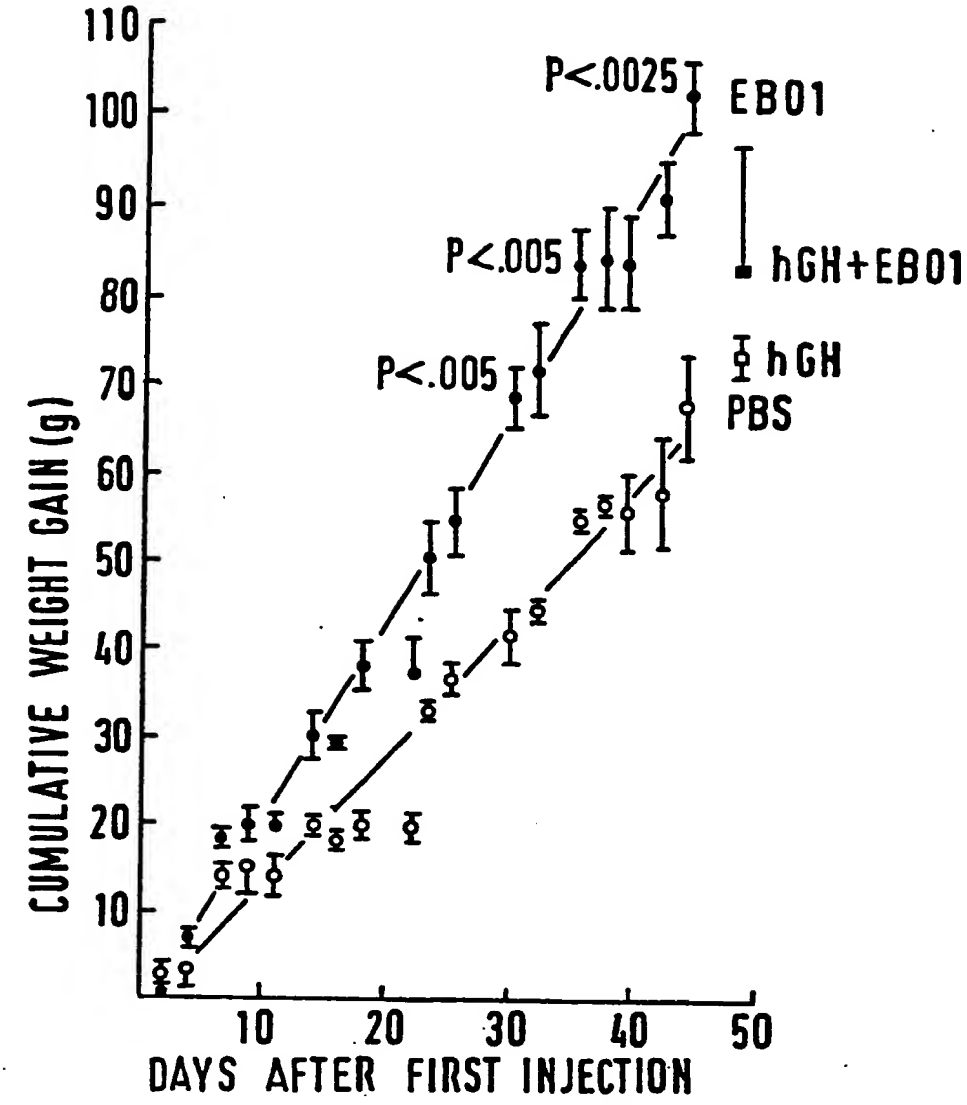

FIG.4

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(54) Physiologically active compositions.

(57) It has previously been shown that bivalent antibodies to insulin can mimic the action of insulin in vivo. However antibodies in vivo were believed to have the opposite effect.

It has now been found that the administration to vertebrates of certain specific anti-hormone antibodies, preferably monoclonal antibodies, with or without administration of the hormone itself, can potentiate the biological action of the hormone. Active immunisation with a fragment of the hormone can also be carried out. Such methods can be used therapeutically or alternatively to increase the response of the vertebrate to the hormone beyond normal physiological levels. When the hormone is growth hormone, accelerated growth of economically important animals can be achieved.

EP 0 137 234 A3

European Patent Office

EUROPEAN SEARCH REPORT

0137234

Application number

EP 84 10 9773

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Y	CHEMICAL ABSTRACTS, vol. 98, no. 7, 14th February 1983, page 497, abstract no. 51671a, Columbus, Ohio, US; J. IVANVI: "Study of antigenic structure and inhibition of activity of human growth hormone and chorionic somatomam motropin by monoclonal antibodies", & MOL. IMMUNOL. 1982, 19(12), 1611-18 * Abstract *	1	A 61 K 39/395 A 61 K 37/24 A 61 K 39/00 // C 12 P 21/00 C 12 N 15/00
Y	CHEMICAL ABSTRACTS, vol. 97, no. 5, 2nd August 1982, pages 57,58, abstract no. 33510x, Columbus, Ohio, US; L.A. RETEGUI et al.: "Monoclonal antibodies against growth hormone: effects on the hormone interaction with specific cell surface receptors", & PROTIDES BIOL. FLUIDS 1981 (Pub. 1982) 29th, 827-32 * Abstract *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 61 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27-05-1987	REMPP G.L.E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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